(For Laboratory Use Only)
Accuratus Lab Services Project A21009

SRW 6/23/16

Test substance tracking #:
TS010416.SRC46
14614 5-16-16

ACCURATUS LAB SERVICES

PROTOCOL

AOAC Fungicidal Activity Method

Test Organism:

Trichophyton mentagrophytes (ATCC 9533)

PROTOCOL NUMBER

SRC46121515.FACT.2

PREPARED FOR

CID LINES nv
Waterpoortstraat 2
Ieper 8900
Belgium

SPONSOR REPRESENTATIVE

Scientific & Regulatory Consultants, Inc.
201 W. Van Buren Street
Columbia City, IN 46725

PERFORMING LABORATORY

Accuratus Lab Services
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

DATE

December 15, 2015

EXACT COPY
INITIALS JLH DATE 7-5-16

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PURPOSE

The purpose of this study is to determine the effectiveness of the Sponsor's product as a disinfectant for hard surfaces following the AOAC Fungicidal Activity of Disinfectants method. This method is in compliance with the requirements of the following: The U. S. Environmental Protection Agency (EPA).

TEST SUBSTANCE CHARACTERIZATION

According to (40 CFR, Part 160, Subpart F [160.105]) test substance characterization as to identity, strength, purity, solubility and composition, as applicable, shall be documented before its use in this study. The stability of the test substance shall be determined prior to or concurrently with this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to Accuratus Lab Services. Accuratus Lab Services will append Sponsor-provided Certificates of Analysis (C of A) to this study report, if requested and supplied. Characterization and stability studies not performed following GLP regulations will be noted in the Good Laboratory Practice compliance statement.

SCHEDULING AND DISCLAIMER OF WARRANTY

Experimental start dates are generally scheduled on a first-come/first-serve basis once Accuratus Lab Services receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is January 4, 2016. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of February 1, 2016. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at Accuratus Lab Services.

If a test must be repeated, or a portion of it, due to failure by Accuratus Lab Services to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing.

If the Sponsor requests a repeat test, they will be charged for an additional test. Neither the name of Accuratus Lab Services nor any of its employees are to be used in advertising or other promotion without written consent from Accuratus Lab Services.

The Sponsor is responsible for any rejection of the final report by the regulatory agencies concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the Accuratus Lab Services final report and notify Accuratus Lab Services of any perceived deficiencies in these areas before submission of the report to the regulatory agency. Accuratus Lab Services will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM

Regulatory agencies require that a specific fungal claim for a test substance intended for use on hard surfaces be supported by appropriate scientific data demonstrating the efficacy of the test substance against the claimed fungi. This is accomplished by treating the target organism with the test substance under conditions which simulate as closely as possible, in the laboratory, the actual conditions under which the test substance is designed to be used. For products intended for use on hard surfaces (inanimate environmental surfaces), a suspension method may be used in the generation of the supporting data. The experimental design in this protocol meets these requirements. The test system to be used in this study will follow the AOAC Fungicidal Activity of Disinfectants.

TEST PRINCIPLE

A suspension of the test organism is exposed to the test substance for specified exposure time(s). After exposure, the aliquots of the suspension are transferred to vessels containing neutralizing subculture media and assayed for survivors. Appropriate culture purity, sterility, viability, initial suspension population and neutralization confirmation controls are performed. The current version of Standard Operating Procedure CGT-0023 reflects the methods which shall be used in this study.

TEST METHOD

Test Organism	ATCC #	Growth Medium	Incubation Parameters
Trichophyton mentagrophytes	9533	Sabouraud Dextrose Agar	25-30°C, aerobic

The test organism to be used in this study was obtained from the American Type Culture Collection (ATCC), Manassas, VA.

Recovery Agar Medium: Glucose Agar

Preparation of Test Organism

A culture of *Trichophyton mentagrophytes* will be prepared by inoculating a sufficient number of agar plates using a stock culture and incubating at 25-30°C for 10-15 days. The mycelia will be removed from sufficient plates using a sterile device. The mycelia will be transferred to a glass bottle containing beads and sterile saline or saline/Triton Solution (0.85% Saline + 0.05 % Triton X-100) and mixed thoroughly. Alternately, the mycelia may be added to a tissue grinder containing sterile saline or saline/Triton Solution and macerated. The culture will be filtered through sterile gauze to remove hyphal fragments. The conidial concentration will be estimated by counting in a hemacytometer and the culture may be adjusted as necessary. The culture will be standardized, as necessary, to achieve ≥5 x 10^6 conidia/mL. Applicable culture dilutions will be performed using 0.85% Saline.

An organic soil load may be added to the test culture per Sponsor's request.

Preparation of Test Substance

The test substance(s) to be assayed will be used as directed by the Sponsor. If a dilution of the test substance is requested by the Sponsor, the diluted test substance(s) shall be used within three hours of preparation. Five (5) mL of the test substance, at each dilution if applicable, will be aliquotted into the required number of sterile 25 x 150 mm tubes. The tubes will be placed into a water bath at the Sponsor specified temperature, and allowed to equilibrate for ≥10 minutes prior to testing.

Exposure Conditions

A volume of 0.5 mL of the test organism suspension will be added to a tube containing 5 mL of the equilibrated test substance using a sterile pipette. To inoculate the test substance, remove the tube from the water bath and slant slightly. Add the suspension without touching the fluid or tube walls. Agitate the tube gently after adding culture and return to the water bath. Staggered intervals will be used where necessary.

Test System Recovery

At each exposure time, remove the tube from the water bath, insert a sterile 4 mm i.d. loop into the tube, withdraw the sample without touching the tube walls or lip and transfer to 10 mL of neutralizing subculture medium and mix. Repeat the test system recovery procedure for each exposure time from the same tube of inoculated test substance, as applicable. Secondary subculture transfers are recommended. If performed, transfer 1 loopful from the primary neutralizing subculture medium to a 10 mL tube of secondary neutralizing subculture medium and mix.

Incubation and Observation

The subculture tubes will be incubated for 10 days at 25-30°C. The subculture plates will be incubated for 66-76 hours at 25-30°C. Additional incubation may be followed for the subculture plates if growth is hard to detect. Following incubation, the subcultures will be visually examined for growth. If necessary, subcultures can be stored for up to 3 days at 2-8°C prior to examination. Representative subculture tubes demonstrating growth (positive tubes) will be subcultured onto appropriate agar for confirmation of the test organism.

STUDY CONTROLS

Purity Control
A "streak plate for isolation" will be performed on the organism culture and following incubation examined in order to confirm the presence of a pure culture. The acceptance criterion for this study control is a pure culture demonstrating colony morphology typical of the test organism.

Organic Soil Load Sterility Control
The serum used for soil load will be cultured, incubated, and visually examined for growth. The acceptance criterion for this study control is lack of growth.

Viability Control
One loopful of the conidial suspension will be transferred to a tube of neutralization subculture medium, per type used in the study. The inoculated subculture medium will be incubated and visually examined for growth. The acceptance criterion for this study control is growth.

Neutralizing Subculture Medium Sterility Control
A representative sample of uninoculated neutralizing subculture medium will be incubated and visually examined for growth. The acceptance criterion for this study control is lack of growth.

Initial Suspension Population Control
The final test culture will be serially diluted and plated using standard microbiological techniques. Following incubation, the organism plates will be observed to enumerate the concentration of the test organism present at the time of testing. The acceptance criterion for this study is a minimum of 5×10^6 conidia/mL.

Neutralization Confirmation Control
The neutralization of the test substance will be confirmed by transferring 1 loopful of the test substance to a primary tube containing 10 mL of neutralizing subculture medium and mix. If performed in the test procedure, transfer 1 loopful of broth from the primary tube to a 10 mL tube of secondary neutralizing subculture medium and mix. Each subculture tube will be challenged with a low level of the organism to target 10-100 CFU per tube (neutralization control). The aliquots added to the tubes will be plated in duplicate as a numbers control. Multiple organism dilutions may be utilized. If multiple concentrations of test substance are evaluated in the study, only the most concentrated test substance needs to be evaluated in this control. The subcultures will be incubated as in the test. The acceptance criterion for this control is growth in the final subculture medium, minimally, with ≤100 CFU added per tube.

If all the organism dilution(s) used in this control fail to provide adequate numbers (10-100 CFU) which coincides in a failure to meet the acceptance criterion for this study control, the control may be repeated in its entirety.

PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM

Accuratus Lab Services maintains Standard Operating Procedures (SOPs) relative to efficacy testing studies. Efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including test organism strains for purposes of identification, receipt and use of chemical reagents. These procedures are designed to document each step of efficacy testing studies. Appropriate references to medium, batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each efficacy test is assigned a unique Project Number when the protocol for the study is initiated by the Study Director. This number is used for identification of the test subcultures, etc. during the course of the test. Test subcultures are also labeled with reference to the test organism, experimental start date, and test product. Microscopic and/or macroscopic evaluations of positive subcultures are performed in order to confirm the identity of the test organism. These measures are designed to document the identity of the test system.

METHOD FOR CONTROL OF BIAS: N/A

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
The efficacy performance requirements for label claims state that the test substance must kill the conidial spores in all subculture tubes within 10 minutes to be an effective fungicide.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section. If any of the control acceptance criteria are not met, the test may be repeated under the current protocol number.

Any positive test subcultures confirmed as a contaminant will be reported. Any test set that demonstrates contamination may be invalidated per Sponsor's request and may be re-tested.

REPORT

The report will include, but not limited to, identification of the sample, date received, initiation and completion dates, identification of the fungal strains used, description of media and reagents, description of the methods employed, tabulated results and conclusion as it relates to the purpose of the test, and all other items required by 40 CFR Part 160.185.

PROTOCOL CHANGES

If it becomes necessary to make changes in the approved protocol, the revision and reasons for change will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study.

Standard operating procedures used in this study will be the current effective revision at the time of the work, Any minor changes to SOPs (for this study) or methods used will be documented in the raw data and approved by the Study Director.

TEST SUBSTANCE RETENTION

It is the responsibility of the Sponsor to retain a sample of the test substance. All unused test substance will be discarded following study completion unless otherwise requested by Sponsor.

RECORD RETENTION

Study Specific Documents
All of the original raw data developed exclusively for this study shall be archived at Accuratus Lab Services for a minimum of five years for GLP studies or a minimum of six months for all other studies following the study completion date. After this time, the Sponsor (or the Sponsor Representative, if applicable) will be contacted to determine the final disposition. These original data include, but are not limited to, the following:

1. All handwritten raw data for control and test substances including, but not limited to notebooks data forms and calculations.
2. Any protocol amendments/deviation notifications.
3. All measured data used in formulating the final report.
4. Memoranda, specifications, and other study specific correspondence relating to interpretation, and evaluation of data, other than those documents contained in the final study report.
5. Original signed protocol.
6. Certified copy of final study report.
7. Study-specific SOP deviations made during the study.

Facility Specific Documents
The following records shall also be archived at Accuratus Lab Services. These documents include, but are not limited to, the following:

1. SOPs which pertain to the study conducted.
2. Non study-specific SOP deviations made during the course of this study which may affect the results obtained during this study.
3. Methods which were used or referenced in the study conducted.
4. QA reports for each QA inspection with comments.
5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

REFERENCES

1. Association of Official Analytical Chemists (AOAC) Official Method 955.17, Fungicidal Activity of Disinfectants. In Official Methods of Analysis of the AOAC, 1955.
2. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method [Preparation of Synthetic Hard Water]. In Official Methods of Analysis of the AOAC, 2013 Edition.
3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Uses of Antimicrobial Agents, September 4, 2012.
4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Hard Surfaces- Efficacy Data Recommendations, September 4, 2012.

DATA ANALYSIS

Calculations:

$$\text{Conidia/mL} = \frac{\text{(average CFU @ dilution used) x (dilution factor)}}{\text{(volume plated in mL)}}$$

Statistical Methods: None used.

STUDY INFORMATION

(All blank sections are completed by the Sponsor or Sponsor Representative as linked to their signature, unless otherwise noted.)

Test Substance (Name and Batch Number) exactly as it should appear on final report:
Virocid, Lot S514701, Lot S514702

The requirement to test at the lower certified limit (LCL) for registration has not yet been defined for this organism by the U.S. EPA; however, testing at the LCL is recommended due to agency uncertainty.

Product Description:
- ☑ Quaternary ammonia
- ☐ Iodophor
- ☐ Sodium hypochlorite
- ☐ Peracetic acid
- ☐ Peroxide
- ☑ Other Glutaraldehyde①

Approximate Test Substance Active Concentration (upon submission to Accuratus Lab Services):
<2.4% quat., M9.4% glut.
(This value is used for neutralization planning only. This value is not intended to represent characterization values.)

Neutralization/Subculture Broth: ☐ _____
(All neutralizing subculture medium must support the growth of the test organism.)
☑ Accuratus Lab Services' Discretion. By checking, the Sponsor authorizes Accuratus Lab Services, at their discretion, to perform neutralization confirmation assays at the Sponsor's expense prior to testing to determine the most appropriate neutralizer. (See Fee Schedule).

Storage Conditions:
- ☑ Room Temperature
- ☐ 2-8°C
- ☐ Other_____

Hazards:
- ☐ None known: Use Standard Precautions
- ☑ Material Safety Data Sheet, Attached for each product
- ☐ As Follows:_____

Product Preparation
- ☐ No dilution required, Use as received (RTU)
- ☑ *Dilution(s) to be tested:
  1:400 defined as 1 part (amount of test substance) + 399 parts (example: 1 oz/gallon) (amount of diluent)
  - ☐ Deionized water or Autoclave Sterilized)
  - ☐ Soft Tap Water (Filter or Autoclave Sterilized)
  - ☑ AOAC Synthetic Hard Water: 400 PPM
  - ☐ Other _____

***Note: An equivalent dilution may be made unless otherwise requested by the Sponsor.**

Test Organism: ☑ *Trichophyton mentagrophytes* (ATCC 9533)

Exposure Temperature: 20±1°C

Exposure Times: 5 minutes, 9.5 minutes, 15 minutes

Organic Soil Load:
- ☐ Minimum 5% Fetal bovine serum added to conidial suspension
- ☑ No Soil Load Required
- ☐ Other_____

①Added per email. JLH 6-31-16

TEST SUBSTANCE SHIPMENT STATUS
(This section is for informational purposes only.)
☒ Test Substance is already present at Accuratus Lab Services.
☐ Test Substance has been or will be shipped to Accuratus Lab Services.
Date of expected receipt at Accuratus Lab Services:_____
☐ Test Substance to be hand-delivered (must arrive by noon at least one day prior to testing or other arrangements made with the Study director)

COMPLIANCE
Study to be performed under EPA Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures.
☑ Yes
☐ No (Non-GLP or Development Study)

TEST SUBSTANCE CHARACTERIZATION & STABILITY TESTING
[Verification required per 40 CFR Part 160 Subpart B (160.31(d))].

☐ Characterization/Stability testing is not required (For Non-GLP or Development testing only)

OR

Physical and Chemical Characterization (Identity, purity, strength, solubility, as applicable) of the test lots

☑ **Physical & Chemical Characterization has been or will be completed prior to efficacy testing.**

GLP compliance status of physical & chemical characterization testing:
☑ Testing was or will be performed following 40 CFR Part 160 GLP regulations
☐ Characterization has not been or will not be performed following GLP regulations

Check and complete the following that apply:
☑ A Certificate of Analysis (C of A) has been or will be provided for each lot of test substance to be appended to the report.
☐ Testing has been or will be conducted at Accuratus Lab Services under protocol or study #:
_____
☐ Test has been or will be conducted by another facility under protocol or study #:
_____

☐ **Physical & Chemical Characterization was not or will not be performed prior to efficacy testing.**

Stability Testing of the formulation

☑ **Stability testing has been or will be completed prior to or concurrent with efficacy testing.**

GLP compliance status of stability testing:
(GLP compliance is required by 40 CFR Part 160)
☑ Testing was or will be performed following 40 CFR Part 160 GLP regulations
☐ Stability testing has not been or will not be performed following GLP regulations

Check and complete the following that apply:
☐ Testing has been or will be conducted at Accuratus Lab Services under protocol or study #:
_____
☐ Test has been or will be conducted by another facility under protocol or study #:
_____

☐ **Stability testing was not or will not be performed prior to or concurrent with efficacy testing.**

If test substance characterization or stability testing information is not provided or is not performed following GLP regulations, this will be indicated in the GLP compliance statement of the final report.

PROTOCOL MODIFICATIONS

☐ Approved without modification
☑ Approved with modification
The subculture broths will not be refrigerated after incubation. A draft report will be provided for review prior to finalization.

PROTOCOL ATTACHMENTS

Supplemental Information Form Attached - ☐ Yes ☑ No

APPROVAL SIGNATURES

SPONSOR:

NAME: Ms. Rhonda Jones TITLE: Agent

SIGNATURE: [signature] DATE: 5-13-16

PHONE: (260) 244 - 6270 FAX: (260) 244 - 6273 EMAIL: rjones@srcconsultants.com

For confidentiality purposes, study information will be released only to the sponsor/representative signing the protocol (above) unless other individuals are specifically authorized in writing to receive study information.

Other individuals authorized to receive information regarding this study: ☐ See Attached

Delphine Scopinski, Helene Charier, SRC Staff

Accuratus Lab Services:

NAME: Jamie Herzan
Study Director

SIGNATURE: Jamie Herzan DATE: 5-31-16
Study Director